Raggin' on T-bet

Richard M. Locksley[1,*]
[1]HHMI and Departments of Medicine and Microbiology and Immunology, University of California, San Francisco, San Francisco, CA 94143-0795, USA
*Correspondence: locksley@medicine.ucsf.edu
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Although increasing adiposity is usually associated with adipose tissue infiltration by inflammatory cells and systemic insulin resistance, Stolarczyk et al. (2013) report in this issue that mice deficient in the T-box transcription factor, T-bet, display a dissociated phenotype with enhanced perigonadal adipose but enhanced insulin sensitivity.

The last decade has witnessed much progress in characterizing the metabolic syndrome as a dysregulated state associated with chronic systemic inflammation and loss of tissue insulin sensitivity. Not infrequently, increased nutrient storage results in focal adipose damage and infiltration by inflammatory cells, thus setting up a feed-forward cycle whereby insulin insensitivity and adipose tissue inflammation become locked in a metabolically nonfunctional state. T-bet was identified as a transcription factor demonstrated to play a nonredundant role in the differentiation of naive CD4 T cells to the IFN-γ-producing Th1 subset (Szabo et al., 2000). The restricted expression of T-bet, or Tbx21, to the hematopoietic system promised a straightforward interrogation of the role for IFN-γ-expressing inflammatory cells in metabolic homeostasis. In this issue of *Cell Metabolism*, Stolarczyk et al. reveal a relationship between inflammation, adiposity, and insulin insensitivity in T-bet-deficient mice fed a high-fat diet (Stolarczyk et al., 2013).

Stolarczyk et al. found that T-bet-deficient mice gained weight on both regular chow and high-fat diet that could be attributed to increased perigonadal, but not subcutaneous, adipose mass. Despite the increased adiposity, however, insulin sensitivity was increased, and this was accompanied by diminished numbers of hematopoietic cells in perigonadal adipose tissue and a decrease in cytokines released from adipose tissue ex vivo. In order to separate contributions by T-bet in the adaptive and innate immune system, the authors assessed T-bet-deficient mice on a Rag-deficient background that lacks T and B cells, but has persisting NK cells and other innate lymphoid cells that express T-bet. Here, the absence of T-bet conferred no differences in body weight, adiposity, or insulin sensitivity, although the decline in amounts of IFN-γ released from perigonadal adipose tissue persisted, but established adaptive immune cells as being responsible for the phenotype. Adoptive transfer of T-bet-deficient CD4 T cells into Rag-deficient mice recapitulated a partial phenotype: mice showed modestly enhanced insulin sensitivity and had decreased numbers of adipose CD4 T cells. Finally, IFN-γ, a known target of T-bet, was incriminated by the observation that crossing T-bet- and IFN-γ-deficient mice largely abrogated the phenotype.

Thus, T-bet deficiency dissociates perigonadal adiposity from insulin sensitivity, and this is dependent on adaptive immunity and at least partially on CD4 T cells and IFN-γ. As the authors point out, T-bet-deficient mice had increased numbers of $Foxp3^+$ regulatory CD4 T cells, or Tregs, in perigondadal adipose tissue as compared to wild-type mice. Recent reports have called attention to Tregs in adipose tissue, where they have tissue protective and insulin-promoting effects by virtue of suppressing inflammatory cell accumulation and activation. Interestingly, adipose Tregs express genes involved in lipid synthesis and storage and respond to agonistic PPARγ thiazolidinedione drugs, suggesting these cells may contribute directly to adipose storage while suppressing cytokine production by immune effector cells (Cipolletta et al., 2012). This phenotype mirrors the phenotype in the T-bet knockout mice and would be additionally consistent with the loss of the phenotype upon crossing to the Rag-deficient background, which lack all T cells, including Tregs. This would also explain the partial reconstitution of the phenotype by adoptive transfer of CD4 T cells into Rag-deficient mice, although the authors do not examine the numbers of $Foxp3^+$ Tregs that repopulate the perigonadal adipose tissue.

A puzzling aspect of the Treg explanation is the IFN-γ-dependent nature of the phenotype. Tregs are suppressor cells and do not typically produce cytokines like IFN-γ, but the results suggest that Tregs are reading out IFN-γ-dependent signals necessary for their functional role in mediating adipose storage and immune cell suppression. How might this be?

Increasing evidence suggests that Tregs come in flavors that mirror features of the effector CD4 helper T cell subsets they suppress (Chaudhry and Rudensky, 2013). From a simplified mechanistic point of view, Tregs need to integrate the same overlapping sets of environmental cues, including chemokines and cytokines, used by effector T cell subsets in order to accumulate in the tissues and immune microenvironments where regulation is needed to suppress otherwise damaging autoreactive responses under diverse immunologic conditions. Like T-bet in Th1 cell differentiation, GATA-3 is intricately involved in the epigenetic programming mediating Th2 development; evidence for direct interaction of T-bet in suppressing GATA-3 levels during T cell differentiation likely contributes to the biasing of T-bet-deficient cells to express Th2-associated cytokines under some conditions (Hwang et al., 2005). Thus, in the absence of T-bet, GATA-3 expression is left unrestrained in T cells. Intriguingly, GATA-3 is highly expressed in adipose Tregs, where expression correlates directly with PPARγ (Cipolletta et al.,

2012), consistent with the increased numbers of Foxp3$^+$ adipose Tregs in T-bet-deficient mice.

So how might these cells be dependent upon IFN-γ for their metabolic phenotype? In naive T cells, IFN-γ from other cells (or IL-27, which shares this capacity) induces T-bet, which transcriptionally targets the second chain of the IL-12 receptor (the first is constitutive), enabling these cells to use IL-12 to super-induce IFN-γ production through Stat1/Stat4-facilitated epigenetic reprogramming of the IFN-γ locus. In Tregs, T-bet is expressed in the first step, but expression of IL-12Rβ2 is not, in part explained by epigenetic silencing of the locus, thus depriving Tregs of effector cytokines while leaving them programmed to sense inflammatory mediators (Koch et al., 2012). Such effects may be amplified in the genetic absence of T-bet. In adipose tissues, where GATA-3 is highly expressed in Tregs and more readily induced in the absence of T-bet, robust Tregs may facilitate central adipose storage while suppressing hematopoietic cell infiltration, as seen by Stolarczyk et al. In peripheral tissues, residual IFN-γ driven by eomesodermin (Tbr2, a Tbox21 cousin) in T and NK cells (Pearce et al., 2003) may be the source of T-bet-independent signals that sustain Tregs in a state such that peripheral manifestations of inflammation, which can occur at mucosal sites in T-bet-deficient mice (Garrett et al., 2007), are abrogated, thus promoting systemic insulin sensitivity. Indeed, the authors point out that their immunodeficient mouse colonies are devoid of the colitogenic microbial flora that drives inflammation in some of these knockout mice, perhaps reflecting a role for T-bet in sustaining mucosa-protective IL-22-producing innate lymphoid cells at the expense of IL-17- and IFN-γ-dependent pathology (Buonocore et al., 2010), while providing protection against epithelial pathogens (Klose et al., 2013). Indeed, when crossed to the T-bet-deficient background, Rag-deficient mice lost some but not all IFN-γ production, revealing a basal state that may be dependent on the environment in which the mice are maintained. How Tregs and ILCs interact is a highly topical question, and the authors' model may provide one system for such investigation.

The findings of Stolarczyk et al. highlight the continuing surprises unearthed in the young field of "immunometabolism" but underscore the need for consideration of the integrative nature of metabolism in peeling away the multiple layers of overlying regulation, including by cells and cytokines, of the immune system, both adaptive and innate.

REFERENCES

Buonocore, S., Ahern, P.P., Uhlig, H.H., Ivanov, I.I., Littman, D.R., Maloy, K.J., and Powrie, F. (2010). Nature *464*, 1371–1375.

Chaudhry, A., and Rudensky, A.Y. (2013). J. Clin. Invest. *123*, 939–944.

Cipolletta, D., Feuerer, M., Li, A., Kamei, N., Lee, J., Shoelson, S.E., Benoist, C., and Mathis, D. (2012). Nature *486*, 549–553.

Garrett, W.S., Lord, G.M., Punit, S., Lugo-Villarino, G., Mazmanian, S.K., Ito, S., Glickman, J.N., and Glimcher, L.H. (2007). Cell *131*, 33–45.

Hwang, E.S., Szabo, S.J., Schwartzberg, P.L., and Glimcher, L.H. (2005). Science *307*, 430–433.

Klose, C.S.N., Kiss, E.A., Schwierzeck, V., Ebert, K., Hoyler, T., d'Hargues, Y., Göppert, N., Croxford, A.L., Waisman, A., Tanriver, Y., and Diefenbach, A. (2013). Nature *494*, 261–265.

Koch, M.A., Thomas, K.R., Perdue, N.R., Smigiel, K.S., Srivastava, S., and Campbell, D.J. (2012). Immunity *37*, 501–510.

Pearce, E.L., Mullen, A.C., Martins, G.A., Krawczyk, C.M., Hutchins, A.S., Zediak, V.P., Banica, M., DiCioccio, C.B., Gross, D.A., Mao, C.A., et al. (2003). Science *302*, 1041–1043.

Stolarczyk, E., Vong, C.T., Perucha, E., Jackson, I., Cawthorne, M.A., Wargent, E.T., Powell, N., Canavan, J.B., Lord, G.M., and Howard, J.K. (2013). Cell Metab. *17*, this issue, 520–533.

Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., and Glimcher, L.H. (2000). Cell *100*, 655–669.